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Abstract: We recently presented a case series of seven children who developed severe and complicated Guillain-Barré syndrome (GBS) after infection with M. pneumoniae (Mp) (Meyer Sauteur et al., 2015). The disease was rapidly progressive and severe: one died, four had clinically defined central nervous system (CNS) involvement, and five required mechanical ventilation.

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Running headline:

Anti-GalC antibodies in severe and complicated *M. pneumoniae*-associated childhood GBS

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Key words

Central nervous system; children; galactocerebroside; Guillain-Barré syndrome; *Mycoplasma pneumoniae*

Dear Editor,

We recently presented a case series of seven children who developed severe and complicated Guillain-Barré syndrome (GBS) after infection with *M. pneumoniae* (*Mp*) (*Meyer Sauteur et al., 2015*). The disease was rapidly progressive and severe: one died, four had clinically defined central nervous system (CNS) involvement, and five required mechanical ventilation. Given the often relatively benign disease course of GBS after this specific type of infection (*Meyer Sauteur et al., 2016*) the severe and complicated GBS in this series was rather unexpected. Since we recently demonstrated that pediatric and adult GBS after *Mp* infection is associated with antibodies against galactocerebroside (GalC) (*Meyer Sauteur et al., 2016*) we investigated if anti-GalC antibodies were also present in these children with severe GBS.

Sera were tested for IgM and IgG antibodies to GM1, GM2, GD1a, GD1b, GQ1b, and GalC (all from Sigma-Aldrich, Zwijndrecht, the Netherlands) as previously described (*Ang et al., 2000; Kuijf et al., 2005; Meyer Sauteur et al., 2016*). CSF was tested for IgM and IgG to GalC at 1:10 dilution. ODs at 490 nm from uncoated wells (containing ethanol) were subtracted from glycolipid-coated wells. Cut-off values (0.05 for IgG and 0.03 for IgM) were obtained by measuring 14 CSF samples of patients with other neurological diseases (mean OD plus 3 times the standard deviation). Some patients had previously been included in other studies (*Meyer Sauteur et al., 2016; Meyer Sauteur et al., 2014; Roodbol et al., 2014*).

Data were analyzed using the R software environment (version 3.4.1). The χ^2 test was used to compare proportions. Two-sided *p* values <0.05 were considered to be statistically significant. The study was approved by the Erasmus MC Medical Ethics Board.

Anti-GalC IgG and/or IgM were found in six out of seven patients (86%). Antibodies against other glycolipids were present in three of those six (anti-GM1). No antibodies were found against complexes of two glycolipids (data not shown).

Since anti-GalC IgG is specifically associated with GBS after *Mp* infection (*Meyer Sauteur et al., 2016*), we next compared the frequency of anti-GalC IgG in severe *Mp*-positive pediatric GBS to (1) *Mp*-positive pediatric GBS patients (cohort as previously described (*Meyer Sauteur et al., 2016*)), who did not fulfill the criteria for severe GBS (*Meyer Sauteur et al., 2015*) (defined as "less-severe" GBS), and (2) *Mp*-negative pediatric GBS (*Meyer Sauteur et al., 2016*). The presence of anti-GalC IgG was significantly more frequent in serum of severe *Mp*-positive GBS patients (43%, n=3/7) than in "less-severe" *Mp*-positive GBS (17%, n=2/12; p=0.04, **Figure 1**) or *Mp*-negative GBS (0%, n=0/8; p=0.03).

Sufficient CSF was available in three patients with severe *Mp*-positive GBS. CSF anti-GalC IgG and IgM was found in three (100%) and one (33%), respectively. All these three did show signs and symptoms of CNS involvement.

The increased presence of anti-GalC IgG in severe *Mp*-positive GBS compared to "less-severe" *Mp*-positive GBS may suggest that these antibodies are also involved in the development of severe and complicated GBS. Anti-GalC IgG was also detected in another recent study in GBS as most frequent anti-glycolipid antibody, identified in 37% (*Kuwahara et al., 2017*). The reason for the poor outcome in our series (one patient died and only two patients recovered completely) remains unclear. We (*Meyer Sauteur et al., 2016*) and Samukawa and coworkers (*Samukawa et al., 2014*) found previously no significant difference in the outcomes between anti-GalC-positive and anti-GalC-negative GBS. In both studies, the anti-GalC-negative

group consisted of different subgroups of GBS patients including *Campylobacter jejuni*-related anti-GM1-positive patients who are known to have a poor outcome (*Rees et al., 1995*). Here, all comparisons were performed within the *Mp*-positive GBS group. Another risk factor for poor outcome in GBS is more severe disease at entry (*van den Berg et al., 2013*). The development of GBS may also depend on patient-related factors that influence the susceptibility to produce cross-reactive anti-glycolipid antibodies triggered by infections (*Huizinga et al., 2015*). The differential outcome between severe *Mp*-positive GBS patients of our series and "less-severe" *Mp*-positive GBS may suggest that our patients were more prone to produce a stronger immune response causing GBS. Indeed, the titers of anti-GalC IgG in these patients were higher compared to "less-severe" *Mp*-positive GBS. Apart from the role of anti-GalC antibodies, also other host factors may account for distinct outcomes in GBS (*Geleijns et al., 2006; van den Berg et al., 2014*).

Of the seven GBS patients in this series, four had CNS involvement (two were comatose). The relatively frequent CNS involvement in neurological diseases associated with *Mp* may be common in children. Kuwahara and coworkers (*Kuwahara et al., 2017*) observed that patients with CNS diseases were younger than those with GBS and variants, and hypothesized that this could possibly be a result of the relatively undeveloped blood-brain barrier in children.

In conclusion, the correlation of anti-GalC IgG with a severe and complicated disease course and presence in CSF of patients with CNS involvement suggests that these antibodies may contribute to the pathogenesis of severe *Mp*-associated childhood GBS.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Figure Legends

Figure 1. Anti-GalC isotype distribution in children with severe and "less-severe" *M. pneumoniae* (*Mp*)-associated GBS. Serum anti-GalC antibodies were determined in severe *Mp*-positive childhood GBS of the case series (*Meyer Sauteur et al., 2015*) and compared to its presence in "less-severe" *Mp*-positive childhood GBS of our previous GBS case-control study (*Meyer Sauteur et al., 2016*). *Mp*+ indicates positive for anti-*Mp* IgM ± IgG (both isotypes have been associated with GBS (*Meyer Sauteur et al., 2016*)). Differences in proportions are indicated with the corresponding *p* value (χ^2 test). **Abbreviations:** GalC, galactocerebroside; GBS, Guillain-Barré syndrome; Ig, immunoglobulin; *Mp*, *M. pneumoniae*.